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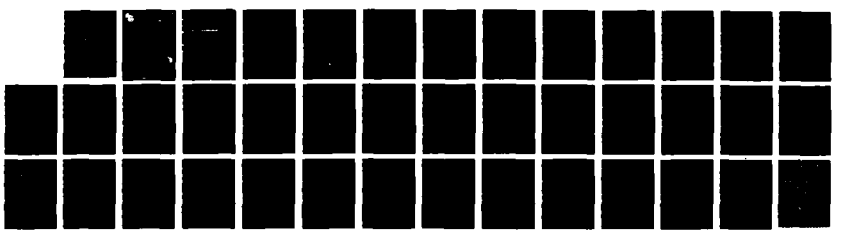

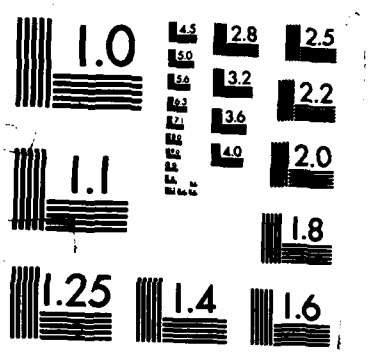
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Institute Report No. 261

Dermal Sensitization Potential of a Schistosome Topical Antipenetrant Lotion in Guinea Pigs

Denzil F. Frost, MS, DVM, CPT VC
Dean K. Magnuson, BS SP4
and
Don W. Korte, Jr, PhD, MAJ, MS

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

March 1988

Toxicology Series: 202

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal sensitization potential of a schistosome topical antipenetrant lotion in guinea pigs (Toxicology Series 202)--Frost et al.

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

for Richard A. Kerstein 5 May 88

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7. AUTHOR(s) Denzil F. Frost, MS, DVM, CPT VC Dean K. Magnuson, BS SP4 Don W. Korte, Jr., PhD, MAJ MS		8. CONTRACT OR GRANT NUMBER(s)
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Dermal Sensitization, Schistosome Topical Antipenetrant Lotion, Guinea Pig Maximization Test, Guinea Pigs

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A schistosome topical antipenetrant lotion, containing 1% niclosamide, was tested for its potential to produce dermal sensitization. The Guinea Pig Maximization Test was used to evaluate dermal sensitization potential. Grade II (mild) dermal sensitization to the lotion was obtained in this study. These results suggest that there is probable risk that the schistosome topical antipenetrant lotion will induce contact sensitivity in humans.

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ABSTRACT

A schistosome topical antipenetrant lotion, containing 1% niclosamide, was tested for its potential to produce dermal sensitization. The Guinea Pig Maximization Test was used to evaluate dermal sensitization potential. Grade II (mild) dermal sensitization to the lotion was obtained in this study. These results suggest that there is probable risk that the schistosome topical antipenetrant lotion will induce contact sensitivity in humans.

Key Words: Dermal Sensitization, Schistosome Topical Antipenetrant Lotion, Guinea Pig Maximization Test, Guinea Pigs

PREFACE

TYPE OF REPORT: Dermal Sensitization GLP Report

TESTING FACILITY:
U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:
U.S. Army Medical Research and Development Command
U.S. Army Medical Materiel Development Activity
Fort Detrick, Frederick, MD 21701-5009

TASK AREA: 808EB Schistosome Topical Prophylaxis System

GLP STUDY NO.: 87016

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ, MS

PRINCIPAL INVESTIGATOR: Denzil F. Frost, MS, DVM, CPT, VC

CO-PRINCIPAL INVESTIGATOR: Dean K. Magnuson, BS, SP4

PATHOLOGIST: Charles B. Clifford, DVM, MAJ, VC, Diplomate, American Society of Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Schistosome Topical Antipenetrant Lotion

INCLUSIVE STUDY DATES: 15 September - 2 November 1987

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of a schistosome topical antipenetrant lotion in guinea pigs.

ACKNOWLEDGMENTS

CPT Gary M. Zaucha, DVM, and SP4 Joel Seewald, BS, provided research assistance; Yvonne C. LeTellier, BS, provided statistical and research assistance; SGT Tammie Heineman, SP4 Barbara Green, and Rick Katona provided animal care and facility management; Ann Wilkinson provided office management during performance of this study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 87016 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 22MAR88

DON W. KORTE, JR., PhD /DATE
MAJ, MS
Study Director

Denzil F. Frost

DENZIL F. FROST, MS, DVM /DATE
CPT, VC
Principal Investigator

Conrad Wheeler 22 MAR 88

CONRAD R. WHEELER, PhD /DATE
DAC
Analytical Chemist

Dean K. Magnuson 22MAR88

DEAN MAGNUSON, BS /DATE
SP4, USA
Co-Principal Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA (70-1n) 15 March 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for Study 87016.

1. I hereby certify that in relation to LAIR GLP Study 87016, the following inspections were made:

31 Aug 1987 - Protocol Review
06 Oct 1987 - Intradermal Induction
13 Oct 1987 - Topical Induction
19 Oct 1987 - Weighing
27 Oct 1987 - Challenge
30 Oct 1987 - 48 Hour Scoring

2. The report and raw data for this study were audited on 28 December 1987.

Carolyn M. Lewis

CAROLYN M. LEWIS
C, QUALITY ASSURANCE

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Dermal Sensitization Potential of a Schistosome Topical Antipenetrant Lotion in Guinea Pigs-Frost *et al*

INTRODUCTION

The U.S. Army Medical Research and Development Command in collaboration with Miles Pharmaceuticals is developing a Topical Antipenetrant (TAP) lotion to protect U.S. military personnel from schistosomiasis. The Division of Toxicology, Letterman Army Institute of Research, was tasked with conducting a preclinical dermal sensitization study of the schistosome TAP. The results of this study will be used in support of the filing of a Notice of Claimed Investigation Exemption for a New Drug (IND) for the Topical Antipenetrant lotion with the U.S. Food and Drug Administration. The Guinea Pig Maximization Test (1) was selected as the test system because it is the most sensitive *in vivo* bioassay for evaluating dermal sensitization (2).

Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of a schistosome topical antipenetrant lotion in guinea pigs.

MATERIALS

Test Substance

Name: Schistosome Topical Antipenetrant Lotion.

Manufacturer/Batch: Miles Pharmaceuticals/JD-10-58.

Active Ingredient: Niclosamide 1% (w/v).

Major Inert Ingredients:

Propylene glycol, USP	47.9%
Alcohol, USP	25.4%
Polyethylene glycol 400, NF	23.9%

Source: Mr. Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC 20307-5100

Vehicle: Propylene glycol (J.T. Baker, Phillipsburg, NJ 08365) was used as the test substance vehicle for intradermal induction doses. The expiration date for this lot (501601) was 1 January 1997.

Other test substance information is presented in Appendix A.

Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)/Lot# 11F05U3

Chemical Abstract Service Registry No.: 97-00-7

Source: Sigma Chemical Company
St. Louis, MO 63178

Vehicle: The vehicle for DNCB was a propylene glycol (3%) and sterile water (97%) mixture. Propylene glycol was from the same lot as for the schistosome TAP intradermal induction doses. Sterile water (Lot No. 01-075-FW, expiration date 1 Feb 89) was obtained from Abbott Laboratories (North Chicago, IL).

Preparation of Stock Positive Control Solution: The DNCB solution was prepared by first adding 30 mg DNCB to 1 ml propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of sterile water was added to give a concentration of 0.1% (w/v). The solution was heated to 50-60°C and vortexed to keep the DNCB in solution.

Other positive control substance information is presented in Appendix A.

Negative Control

Name: Placebo Topical Antipenetrant Lotion

Manufacturer/Batch: Miles Pharmaceuticals/JD-10-50

Major Ingredients:		
	Propylene glycol, USP	47.6%
	Alcohol, USP	25.4%
	Polyethylene glycol 400, NF	23.9%
	Purified water, USP	1.7%

Vehicle: Propylene glycol was used at the same concentration as for the test and positive control portions of the study.

Other placebo information is presented in Appendix A.

Other Study Compounds

Freund's complete adjuvant (FCA) was obtained from Sigma Chemical Company, St. Louis, MO 63178, Lot No. 67F-8834. Propylene glycol, from the same lot as for the Schistosome TAP, was used to prepare the dilutions of FCA.

Sodium lauryl sulfate (SLS) was obtained from the Sigma Chemical Company, St. Louis, MO 63178, Lot No. 116F-0012. Petrolatum, White, USP (Moyco Industries, Philadelphia, PA 19132, Lot No. 6503) was used as the vehicle for the SLS. The SLS was prepared as a 10% concentration in the petrolatum.

Animal Data

Sixty-seven male guinea pigs, Hartley strain (Charles River, Kingston, NY), were used for this study. They were identified individually with ear tags numbered 87E00270 through 87E00336, inclusive. Two animals were selected randomly for quality control necropsy evaluation on receipt. Eight of the animals were used for a pilot study to determine the highest tolerated concentration for the intradermal induction dose, a slightly irritating concentration for the topical induction dose, and a non-irritating concentration for the topical challenge dose. Animal weights on receipt (16 Sep 87) ranged from 147 to 282 g. Additional animal data are presented in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow, Diet #5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water, purified by reverse osmosis, was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 17.8°C to 24.4°C and relative humidity in a range of 35 to 52% with occasional spikes as high as 74 % during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-112, "Guinea Pig Maximization Test" (3), which was based on the method of Magnusson and Kligman (1).

Group Assignment/Acclimation

The guinea pigs were quarantined for 10 days. In addition, 11 days were required for the completion of pilot studies. During the quarantine period, the guinea pigs were checked daily for signs of illness and weighed once a week. Nineteen animals were assigned to each of three groups by a stratified randomization technique based on their body weights.

Dosage Levels

The results of the pilot studies indicated that intradermal injections of the schistosome TAP, the stock DNCB solution, the placebo TAP, and the FCA produced necrosis and sloughing of the skin around the injection sites. The maximum tolerated doses for intradermal injections of the schistosome TAP, placebo TAP, DNCB, and FCA were determined to be a 5% concentration of each stock solution.

The stock solution of the schistosome TAP, DNCB, and placebo TAP were shown to be non-irritating when administered topically. Therefore, for the topical induction and the challenge doses, these compounds were administered as the undiluted stock solutions.

Compound Preparation

Intradermal Induction:

A 5% solution of the schistosome TAP was prepared by diluting 0.3 ml of the stock lotion with 5.7 ml of propylene glycol. Freund's complete adjuvant (5%) was prepared by mixing 1.8 ml of the stock FCA with 32.4 ml propylene glycol. A 5% solution of the placebo TAP was prepared by diluting 0.3 ml of stock lotion with 5.7 ml propylene glycol. The DNCB intradermal injection solution was prepared by diluting 0.3 ml of the stock 0.1% DNCB solution with 5.7 ml of propylene glycol. The FCA (5%) and schistosome TAP (5%) combination was prepared by adding 0.2 ml schistosome TAP and 0.2 ml FCA to 3.6 ml of propylene glycol. The FCA (5%) and DNCB (5%) combination was prepared by adding 0.2 ml of DNCB 0.1% solution and 0.2 ml FCA to 3.6 ml of propylene glycol. The FCA (5%) and placebo (5%) combination was prepared by adding 0.2 ml of placebo and 0.2 ml FCA to 3.6 ml of propylene glycol.

Topical Induction and Challenge:

The sodium lauryl sulfate (SLS) was prepared by weighing 10 g SLS and 90 g petrolatum. The two compounds were hand mixed with a spatula.

The schistosome TAP topical patch was prepared by applying lotion to a 2 x 4 cm piece of Whatman #2 filter paper (Whatman, Ltd, New England). The DNCB topical patch was prepared by applying 0.5 ml of DNCB 0.1% solution to a 2 x 4 cm piece of Whatman #2 filter paper. DNCB solutions were prepared fresh for each application day. The placebo TAP topical patch was prepared by applying 0.5 ml of the placebo lotion to a 2 x 4 cm piece of Whatman #2 filter paper. The challenge vehicle control topical patch for the DNCB group was prepared by mixing 1 ml propylene glycol with 29 ml sterile water (Abbott Laboratories, North Chicago, IL 60064).

Test Procedures

Intradermal Injections:

Twenty-four hours before intradermal dosing, an area of 4 x 6 cm over the shoulder of each animal was clipped (Oster®, Model A5, size 40 blade, Sunbeam Corporation, Milwaukee, WI) and then shaved with a disposable razor (Davol, Inc., Cranston, RI) and tap water.

On each shoulder a row of 3 injections (1 cc syringe, Lot 6 D390, Becton Dickson; 26 gauge, 0.5 in. needle, Becton Dickson), six injections in all, were made as follows: 1) 0.1 ml of the adjuvant alone, 2) 0.1 ml of test substance without adjuvant, and 3) 0.1 ml of the test substance emulsified in FCA (final concentration 5%). All injections were made deep into the dermis to minimize sloughing.

Topical Application:

One week after the injections and 24 hours before the topical application, the 4 x 6 cm area was clipped again and shaved closely with a disposable razor and tap water. Since topical administration of the test agent was non-irritating, the area was pretreated with 10% sodium lauryl sulfate in petrolatum immediately after the clipping and shaving. The SLS was massaged into the skin with a glass rod and the skin was not covered. The residual SLS was removed the next day with gauze just prior to topical induction.

A 2 x 4 cm patch of Whatman No. 2 filter paper was saturated with approximately 0.5 ml of the appropriate test or control substance. The patch was covered by overlapping with an occlusive tape (Blenderm®, Medical Products Division/3M, St. Paul, MN 55144, Lot 273) approximately twice the size of the patch. The occlusive tape was firmly secured by elastic adhesive bandage (Elastoplast®, Belersdorl, Inc., BDF Plaza, Norwalk, CT 06856-5229, Lot 2595). This dressing was left in place for 48 hours. Care was taken to ensure that the wraps remained in place throughout the application period.

Challenge Application:

Test compound and negative control animals were challenged topically with the schistosome TAP lotion 2 weeks after the topical induction. Positive control animals were challenged with DNCB 2 weeks after the topical induction. Hair was removed from a 5 x 5 cm area on each flank by clipping and shaving as for the induction phase. The test substance (approximately 0.5 ml) was applied on a 2 x 2 cm piece of Whatman No. 2 filter paper and sealed to the left flank as for the induction phase. A second filter paper was used to apply an equal volume of the vehicle to the contralateral flank in the same manner as the test substance. The patches and occlusive tape were firmly secured by elastic adhesive bandage (Conform®, The Kendal Company, Hospital Products, Boston, MA 02101, Lot 7233, and Vetwrap®, Animal Care Products/3M, St. Paul, MN 55144). The patches were left in place for 24 hours.

Reading the Challenge Reactions:

The challenge reaction was evaluated 24 hours after removal of the patch. Any irritation produced by the plastic tape would be expected to have subsided by then so that any reaction observed could be attributed to an allergic response. The sites were examined again at 48 hours, after removal of the patch, primarily to detect weak or slowly developing reactions.

Reactions were scored on a 4-point scale: no reaction, 0; scattered mild redness (erythema), 1; moderate and diffuse redness, 2; and intense redness and swelling, 3. A response was positive if it exceeded the response on the contralateral side and there was no more than a slight redness attributable to the test substance in the negative control group.

Necropsy

All guinea pigs were submitted for a complete gross necropsy at the conclusion of the 27-day observation period.

Duration of Study

A historical listing of study events appears in Appendix C.

Deviations from Protocol

Shaving was done with disposable razors and water rather than an electric razor in order to obtain a more satisfactory clean-shaven area. Final study group numbers were affected by the need for additional pilot animals (i.e., one was taken from each group) and unexpected deaths due to complications stemming from diarrhea (see Appendix E, Pathologist's Report). It is believed that these deviations from the protocol did not adversely affect the study results.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Experimental Findings

Table 1 summarizes the incidence of reactions 24 and 48 hours after each challenge dose. The schistosome TAP lotion produced a positive response, peaking at 48 hours with a 25% incidence.

Table 2 summarizes the severity of skin reactions at 24 and 48 hours. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. This produced a peak severity index of .25 at 48 hours for schistosome TAP lotion.

Table 1

Incidences of Challenge Skin Reactions

Test Group	Hrs After Challenge 24	48
Schistosome TAP	.19	.25
DNCB Positive Control	.81	.94
Placebo TAP	.11	.00

Table 2

Average Severity of Challenge Skin Reactions

Test Group	Hrs After Challenge 24	48
Schistosome TAP	.25	.25
DNCB Positive Control	1.12	1.44
Placebo TAP	.11	.00

Dinitrochlorobenzene (DNCB) produced a positive response at both time points, peaking at 48 hours. Eighty-one percent of the DNCB-treated animals exhibited a response 24 hours following the challenge doses. These reactions persisted, yielding positive effects in 94% of the animals at 48 hours after dosing. The severity scores for the responses to the challenge doses of DNCB were 1.12 at 24 hours and 1.44 at 48 hours. At 24 hours, 11% of the negative control animals (challenge dose of niclosamide lotion) exhibited a reaction. Individual severity scores are tabulated in Appendix D.

Pathology

Nine animals were removed from the study because of spontaneous death. Gross and microscopic lesions observed in animals dying were consistent with enterocolitis, probably due to *Escherichia coli*. No gross lesions were observed in any of the animals that remained in the study.

DISCUSSION

Guinea Pig Maximization Test

The Guinea Pig Maximization Test is more sensitive than the Landsteiner-Draize or other experimental methods of identifying contact allergens (2). The number of sensitized animals in the test group is an indication of the potency of the contact allergen. Positive patch test reactions may vary considerably in strength. However, it is the total number of positive responses (frequency) and not the intensity which determines the study outcome. The potency of an allergen is based on the percentage of animals sensitized (frequency) according to the following scale (1):

0-8% Weak (Grade I)
9-28 % Mild (Grade II)
29-64% Moderate (Grade III)
65-80% Strong (Grade IV)
81-100% Extreme (Grade V)

If more than 10% of the animals are sensitized (Grade II, Mild), there is an obvious risk that sensitization will occur in humans.

Schistosome Topical Antipenetrant Lotion

In the present study, a schistosome TAP lotion containing 1% niclosamide was evaluated for dermal sensitization potential according to the Guinea Pig Maximization Test criteria (1). The study results show that the schistosome TAP lotion sensitized 25% of animals; this places the lotion in the mild sensitizer category. However, the placebo TAP lotion was also shown to be a mild sensitizer since 11% of the placebo animals exhibited a positive response to the challenge dose. The use of the complex lotion formulations complicate interpretation of the study, especially since the formulations contained 25% alcohol (Appendix A). This is in excess of the recommended maximum concentration of alcohol when it is used as a vehicle for the Guinea Pig Maximization Test (4). However, without additional data (to include the sensitizing potential of the active ingredient, niclosamide), any conclusion except that the two lotions are mild sensitizers is speculative.

Wahlberg and Fregent have emphasized that one should assess the sensitizing potential of a formulated product such as the schistosome TAP lotion by conducting the Guinea Pig Maximization Test on its individual constituents (4), the rationale being that the formulation may prevent one from

obtaining a sufficient concentration of the active ingredient to induce a sensitizing response in the test system, resulting in a false negative response or an understatement of the active ingredient's sensitizing potential. Conversely, the formulation may contain sensitizing contaminants or byproducts that would induce a false positive response. Consequently, without accurate knowledge of the sensitizing potential of niclosamide, the active ingredient, it is difficult to interpret the results from this study of the schistosome TAP lotion.

CONCLUSION

The schistosome topical antipenetrant lotion is a mild sensitizer under conditions of this study.

REFERENCES

1. Magnusson B, Kligman AM. The identification of contact allergens by animal assay. The guinea pig maximization test. J Invest Dermatol 1969; 52: 268-276.

2. Klecak G. Identification of contact allergens: predictive tests in animals. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology and pharmacology. Washington, DC: Hemisphere Publishing Corporation, 1977: 305-339.

3. Guinea Pig Maximization Test. LAIR Standard Operating Procedure OP-STX-112, Letterman Army Institute of Research, Presidio of San Francisco, CA, 10 September 1987.

4. Wahlberg JE, Fregent S. Guinea pig maximization test. In: Maibach HI and Lowe NJ, eds. Models in dermatology, Vol 2. Basel: Karger 1955; 225-233.

Appendix A: CHEMICAL DATA

TEST COMPOUND FORMULATION

Name: Schistosome Topical Antipenetrant Lotion

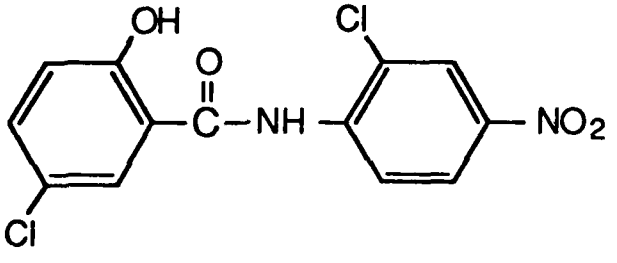
Alternate Name: 1% Niclosamide Lotion

Manufacturer/Batch: Miles Pharmaceuticals/JD-10-58

LAIR Test Code Number: TP82

WRAIR Chemical Number/Bottle Number: WR46,234AJ/BL44970

Active Ingredient:
Niclosamide
Alternate Chemical Names: 2',5-Dichloro-4'-nitro salicylanilide; 5-Chloro-N-(2-chloro-4-nitro phenyl)-2-hydroxybenzamide
Chemical Abstracts Service registry number: 50-65-7
Chemical Structure:



Molecular Formula: $C_{13}H_8Cl_2N_2O_4$

Inert Ingredients: See attached Miles Pharmaceuticals datasheet.

PLACEBO FORMULATION

Name: Placebo Topical Antipenetrant Lotion

Alternate Name: Placebo for Niclosamide Solution

Manufacturer/Batch: Miles Pharmaceuticals/JD-10-50

LAIR Test Code Number: TP83

WRAIR Chemical Number/Bottle Number: WR46,234AK/BL44989

Ingredients: See attached Miles Pharmaceuticals datasheets.

Appendix A (cont.): CHEMICAL DATA

ANALYTICAL DATA

Manufacturer's Analysis: See attached certificates of analysis from Miles Pharmaceuticals.

USAMRDC Analysis: A confirmatory analysis of the test compound and placebo was performed by SRI International.*

*Mammori E, Benitez A, Lim P. Assay of 5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (Niclosamide) in the formulated solutions, WR-46234AJ, BL44970 (1% w/v Active) and WR-46234AK, BL44989 (Placebo). Menlo Park, CA: SRI International, 4 September 1987, Report No. 586; USAMRDC Contract No. DAMD17-85-C-5141.

Appendix A (cont.): CHEMICAL DATA

Niclosamide 1% Solution

Ingredient	Quantity in Grams per Liter: Active	Placebo
Niclosamide	10.00	--
Propylene Glycol USP	479.0	475.5
Alcohol, USP	254.00	254.00
Polyethylene Glycol 400 NF	239.00	239.00
Carbopol 941[1]	5.00	5.00
Tetra (2-hydroxypropyl) ethylene-diamine (Quadrol[2])	5.00	5.00
Purified Water, USP	5.00	17.13
Citric Acid, USP	--	1.30
D&C Yellow #10 Dye	--	0.04
FD&C Red #40 Dye	--	0.03

1 - BF Goodrich Company

2 - BASF Corporation

Appendix A (cont.): CHEMICAL DATA

Miles

MILES

INDUSTRIAL PROPRIETARY DESIGNATION

Miles Pharmaceuticals
Division of Miles Laboratories, Inc

400 Morgan Lane
West Haven, Connecticut 06516
203: 934-9221

June 22, 1987

CERTIFICATE OF ANALYSIS

Product: Niclosamide 1% (w/v) Solution
Miles Batch Number: JD-10-58

TEST	RESULTS
1. Appearance	Bright yellow, viscous, slightly turbid solution.
2. pH	Average = 6.84
3. Specific Gravity	Average = 0.995
4. Assay	Average = 0.979% w/v
5. Degradation Products	5-chlorosalicylic acid: less than 0.01% w/w
	2-chloro-4-nitroaniline: average = 0.025% w/w
6. Viscosity	370 cps
7. Ethanol	Average = 23.56% w/w

I certify that the above data are accurate.

Watson A. Young, Jr.
Supvr., Q.A. Records & Auditing

Appendix A (cont.): CHEMICAL DATA

Miles

MILES

INDUSTRIAL PROPRIETARY DESIGNATION

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.

400 Morgan Lane
West Haven, Connecticut 06516
(203) 934-9221

June 22, 1987

CERTIFICATE OF ANALYSIS

Product: Placebo for Niclosamide Solution
Miles Batch Number: JD-10-50

TEST	RESULTS
1. Appearance	Bright yellow, viscous, slightly turbid solution.
2. pH	Average = 6.91
3. Specific Gravity	Average = 0.993
4. Identity	No niclosamide detected.
5. Viscosity	460 cps
6. Ethanol	Average = 24.07% w/w

I certify that the above data are accurate.

Watson A. Young, Jr.
Supvr., Q.A. Records & Auditing

Appendix A (cont.): CHEMICAL DATA

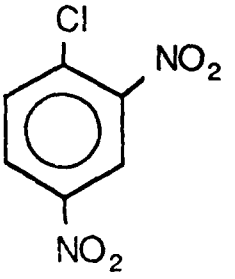
POSITIVE CONTROL

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52-54° C[1]

Purity:

The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[2] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (δ).[3] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[4]

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[5] Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ($CDCl_3$): δ 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[6]

Appendix A (cont.): CHEMICAL DATA

GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[7]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

---

[1]Windholz M, ed. The Merck Index. 5th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

[2]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]*Ibid.* pp 11-12.

[4]*Ibid.* pp 13-16.

[5]Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[6]Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[7]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: Cavia porcellus

Strain: Hartley

Source: Charles River
Kingston, NY

Sex: Male

Date of birth: 24 August 1987

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 20 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tagging procedure, tag numbers 87E00270 to 87E00336, inclusive

Pretest conditioning: Quarantine/acclimation 15 Sep-6 Oct 87

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF EVENTS

Date	Event
15 Sep 87	Sixty-seven animals arrived, were examined, placed in cages, and fed.
16 Sep 87	Animals ear-tagged and weighed.
18 Sep 87	Two animals submitted to necropsy as quality controls.
16 Sep-1 Nov 87	Animals checked daily.
16,18,21,23 Sep 5,19,26 Oct, 2 Nov 87	Animals weighed.
21 Sep 87	Five pilot animals randomly selected. Three pilot animals clipped and shaved for topical trial. Remaining 2 pilots clipped and shaved for 20-100% interdermal trial.
22 Sep 87	Pilot doses prepared. Two pilots dosed intradermally only. Remaining 3 pilots dosed topically only.
24 Sep 87	Topical application dressing and remaining chemical removed. Remaining test animals randomized into groups.
25, 26 Sep 87	Topical pilot animals scored at 24 and 48 hr.
25 Sep 87	Remaining animals removed from quarantine.

Appendix C (cont.): HISTORICAL LISTING OF EVENTS

28 Sep 87	Intradermal pilot animals scored at 6 days. Three pilot animals clipped and shaved for 1-5% intradermal trials.
5 Oct 87	One to 5% intradermal pilot animals scored at 6 days.
5,11,26 Oct 87	All animals clipped and shaved. Doses prepared.
6 Oct 87	Doses prepared. All animals given intradermal induction doses.
12 Oct 87	All animals receive SLS (10%) at test site after being clipped and shaved.
13 Oct 87	Doses prepared. All animals cleaned of residual SLS and given topical induction doses.
15 Oct 87	Topical induction dressing and remaining chemical removed.
27 Oct 87	Doses prepared. All animals given challenge doses.
28 Oct 87	Challenge dose dressing and remaining chemical removed.
29,30,31 Oct 87	All animals scored for 24, 48, and 72 hr skin reactions.
1 Nov 87	Food removed.
2 Nov 87	All animals delivered to Necropsy Suite for sacrifice and gross necropsy.

Appendix D: INDIVIDUAL DERMAL SCORES

Schistosome Topical Antipenetrant

	Hours Post-Challenge	
Animal Number	24	48
(87E00---)		
277	0	1
282	0	0
283	0	0
290	0	0
291	1	0
295	0	0
299	0	0
300	0	0
302	0	0
314	2	1
317	0	0
322	0	0
326	0	0
328	0	1
329	1	1
334	0	0
Incidence of Reaction	.19	.25
Average Severity	.25	.25

Appendix D (cont.): INDIVIDUAL DERMAL SCORES

DNCB Positive Control Group

Animal Number (8722----)	Hours Post-Challenge 24	48
272	2	2
280	1	1
284	2	2
286	1	1
296	1	2
303	1	2
304	2	2
305	1	2
306	0	1
307	0	1
312	1	2
316	1	1
323	0	1
325	2	0
330	2	1
333	1	2
Incidence of Reaction	.81	.94
Average Severity	1.12	1.44

Appendix D (cont.): INDIVIDUAL DERMAL SCORES

Placebo Topical Antipenetrant

	Hours Post-Challenge	
Animal Number	24	48
(87E00---)		
274	0	0
276	0	0
285	0	0
287	0	0
288	0	0
294	1	0
297	0	0
308	0	0
309	0	0
310	0	0
311	0	0
315	1	0
318	0	0
320	0	0
321	0	0
331	0	0
335	0	0
336	0	0
Incidence of Reaction	.11	0
Average severity	.11	0

Appendix E: PATHOLOGY REPORT

Pathology Report GLP 87016

Maximization Test

I. Compound: Niclosamide
Species: Cavia porcellus, Hartley, young adult, male

II. Principal Investigator: CPT Denzil F. Frost
Mammalian Toxicology
Pathologist: MAJ Charles B. Clifford

III. Comment: No internal gross lesions were observed in animals surviving until the termination of the study. Gross and microscopic lesions observed in animals dying prior to scheduled sacrifice are consistent with enterocolitis leading to diarrhea, dehydration, inappetence, and death. Escherichia coli, cultured from the small intestine of two animals, is considered a likely cause.

Charles B Clifford

CHARLES B. CLIFFORD, DVM
MAJ, VC
Division of Pathology

16 November 1987/dbj

Appendix E (cont.): PATHOLOGY REPORT

Attachment:

GROUP : 57 animals, all male, 49 sacrificed 2 Nov 87

ANIMAL ID#	LAIR ID#	GROSS OBSERVATIONS
87E00272	41972	No lesions recognized
274	41973	No lesions recognized
276	41974	No lesions recognized
277	41975	No lesions recognized
280	41976	No lesions recognized
282	41977	No lesions recognized
283	41978	No lesions recognized
284	41979	No lesions recognized
285	41980	No lesions recognized
286	41981	No lesions recognized
287	41982	Fecal staining of hocks. Emaciation.
288	41983	No lesions recognized
290	41984	No lesions recognized
291	41985	No lesions recognized
294	41986	No lesions recognized
296	41987	No lesions recognized
297	41988	No lesions recognized
299	41989	No lesions recognized
300	41990	No lesions recognized
302	41991	No lesions recognized
303	41992	No lesions recognized
304	41993	No lesions recognized
305	41994	No lesions recognized
306	41995	No lesions recognized
307	41996	No lesions recognized
308	41997	No lesions recognized
309	41998	No lesions recognized
310	41999	No lesions recognized
311	42000	No lesions recognized
312	42001	No lesions recognized
314	42002	No lesions recognized
315	42003	No lesions recognized
316	42004	No lesions recognized
317	42005	No lesions recognized
318	42006	No lesions recognized
320	42007	No lesions recognized
321	42008	No lesions recognized
322	42009	No lesions recognized
323	42010	No lesions recognized
325	42011	No lesions recognized
326	42012	No lesions recognized
328	42013	No lesions recognized
329	42014	No lesions recognized
330	42015	No lesions recognized
331	42016	No lesions recognized
333	42017	No lesions recognized
334	42018	No lesions recognized

Appendix E (cont.): PATHOLOGY REPORT

335	42019	No lesions recognized
336	42020	No lesions recognized

Spontaneous Deaths: 8 animals

ANIMAL ID#	LAIR ID#	NECROPSY DATE	GROSS OBSERVATIONS
87E00289	41796	12 Oct 87	Serosanguinous material in the small #1 intestine.
292	41939	29 Oct 87	Emaciation, dehydration.
293	41795	12 Oct 87	Serosanguinous material and gas #2 in the small intestine.
298	41782	5 Oct 87	Severe dehydration, diarrhea. Dark, #3 red-brown material in cecum.
319	41805	19 Oct 87	Pitted, hemorrhagic #4 lesions in stomach. Dark brown contents in cecum.
324	41937	29 Oct 87	*Diminished body fat.*
327	41804	17 Oct 87	Serosanguinous material and gas in the #5 small intestine and cecum.
332	41838	21 Oct 87	Dehydration, emaciation, diarrheal staining of perineum. The intestinal tract was empty except for gas and mucus in the stomach.

#1 1) Mild to moderate, acute, diffuse ileitis, typhlitis, and colitis.
2) Moderate multifocal necrogranulomatous dermatitis. E. coli cultured from small intestine.

#2 1) Moderate, multifocal, proliferative, mycotic esophagitis.
2) Moderate lymphoid depletion, spleen.
3) Minimal cortical vacuolar change, adrenals.
4) Minimal focal myocardial glycogenosis, heart. Culture of small intestinal contents yielded predominantly E. coli.

#3 No microscopic lesions recognized in stomach, small intestine, cecum, or colon. Microbiologic culture of small intestine contents yielded no enteric organisms.

#4 Moderate, acute, diffuse, typhlitis.

#5 Mild, acute, multifocal, ulcerative gastritis.

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